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Effect of Storage Temperature on Germination of Seeds of Twenty Sources of Ponderosa Pine

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Seeds of 20 sources of ponderosa pine (*Pinus ponderosa* var. *scopulorum*), stored for 22 years at 5°C in glass jars, and for 22 years at -16°C in paper sacks, each attained mean germination percentages of 64. Viability among seed sources ranged from 22% to 80% for seed stored at 5°C, and 34% to 83% for seed stored at -16°C. Rate of germination, over all sources, was initially higher through days 4 to 6 for seeds stored at -16°C; however, by day 7 the number of seeds that had germinated was similar for both storage temperatures. Differences between storage temperatures were not consistent among seed sources; for some individual sources, storage at -16°C would be better; for other sources, storage at 5°C would be better. Except for a few sources of relatively low viability in the middle latitudes, there did not appear to be a latitudinal pattern. Also, consistency among sources in their germination response and the relationship of storage at -16°C compared to storage at 5°C did not appear to be related to latitude.

Keywords: Ponderosa pine, seed germination, storage temperature

Management Implications

The availability of viable tree seed is vital to afforestation and reforestation projects to ensure an adequate supply of seed between intermittent crops, and to forest genetics programs to preserve expensive and limited amounts of seed derived from controlled breeding experiments. Development of a reliable long-term seed storage method would meet these requirements.

Introduction

Length of storage and level of temperature (along with proper control of humidity) are important considerations when deciding upon dry storage conditions for seed (USDA Forest Service 1974). Dry seeds of many tree species can be stored at room temperature for short periods or at cool temperatures for longer periods. Some genera whose seeds have stored well for several years or more at temperatures between 0°C and 5°C include *Cupressus*, *Libocedrus*, *Pinus*, *Pseudotsuga*, and *Sequoia* (Barnett and McLemore 1970, Schubert 1954). Schubert (1952, 1954) reported that dry ponderosa pine (*Pinus ponderosa*) seed, stored at 5°C in 5-gallon cans, retained 28% to 94% viability for as long as 20 years; two lots, stored for 17 and 18 years, had viabilities of 75% and 94%, respectively.

However, Barton (1961) emphasized that subfreezing temperatures are optimum for maximum long-term dry storage life of many tree and shrub seeds. Barnett and McLemore (1970) and Schubert and Adams (1971) reported that although temperatures slightly below freezing may be sufficient, seed viability has generally been retained better when seeds were stored at –18°C.

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[1] *The authors, now retired, were at the Station's Research Unit in Lincoln, in cooperation with the University of Nebraska. Station headquarters is in Fort Collins, in cooperation with Colorado State University.*

Materials and Methods

Ponderosa pine (*P. ponderosa* var. *scopulorum*) seeds used in these tests were collected in 1962, 1963, and 1964 for an 80-source Great Plains-wide seed source test (Read 1983, Van Haverbeke 1986). Twenty-four of the geographic sources were collected in 1962 and 1963; 56 sources were collected in 1964. Details of seed collection and handling are given in Read (1980).

The seeds were divided into two groups: one for sowing in a North Dakota nursery, and the other for sowing in a Nebraska nursery. Seeds which were surplus to study needs were stored. The seeds surplus to North Dakota's needs (Bottineau) were stored in a refrigerator at 5°C in sealed glass fruit jars (1/2 pint glass Ken Mason jars; 2 part lid with rubber seal)[2]; the surplus seeds kept in Nebraska (Lincoln) were stored in paper sacks in a freezer at –16°C. When the USDA Forest Service Shelterbelt Laboratory at Bottineau, N. Dak., was closed in 1982, the ponderosa pine seeds stored there in fruit jars were moved to the USDA Forest Service Forestry Sciences Laboratory in Lincoln, Neb., and again were stored at 5°C. At the time of seed germination, moisture content of the seed was uniformly low, averaging 9.5%. During the experiments, seeds were considered germinated when the radicle had elongated to a length equal to or greater than the length of the seed.

Test I

Skepticism regarding the viability of the seeds stored in the glass jars at 5°C prompted a test of germination of all the lots initially stored in North Dakota. Tests were run for 21 days using 100 seeds of each lot, except for five sources where 25 seeds were used because of fewer seeds (table 1). Sixty-eight seed sources were tested; 12 of the 80 sources were not represented in the North Dakota material. This test, in early 1987, dispelled skepticism regarding the viability of the seeds of the various sources. The number of sources in percent germination frequency classes is given below:

Percent germination frequency classes	**No. of sources**
100–91	3
90–81	4
80–71	18
70–61	14
60–51	9
50–41	4
40–31	8
30–21	4
20–11	2
<11	2
Total sources	68

These tests led us to compare the viability of seeds stored at 5°C in glass jars with the viability of seeds stored at –16°C in paper sacks. Three factors sparked

Table 1.—Germination of *Pinus ponderosa* seeds stored for 22 years at 5°C in glass jars.[a]

Seed origin (no.)	Germination[b] (percent)	Seed origin (no.)	Germination (percent)	Seed origin (no.)	Germination (percent)	Seed origin (no.)	Germination[b] (percent)
701	--[c]	766	79	829	82	853	61
702	36	767	20	830	55	854	86
703	--	768	100	831	69	855	72
704	59	811	84	832	83	856	32
720	67	812	94	833	70	857	64
721	51	813	74	834	80	858	--
722	72	814	76	835	72	859	65
723	77	815	77	836	67	860	95
724	38	816	44	837	67	861	73
727	--	817	6	838	75	862	58
753	--	818	12	839	71	863	61
754	40	819	21	840	54	864	61
757	23	820	5	844	27	865	--
758	34	821	46	845	44	866	--
759	51	822	60	846	25	867	--
760	79	823	77	847	34	868	--
761	72	824	70	848	72	869	56
762	60	825	--	849	43		
763	63	826	--	850	35		
764	39	827	67	851	70		
765	--	828	78	852	73		

[a] *Test, one per origin, run for 21 days, January–March, 1987.*
[b] *Basis: 100 seeds (except 25 seeds, origins 702, 767, 768, 811, 869).*
[c] *-- = Seed not available.*

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[2] *Mention of a tradename or priority product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*

interest in such a comparison: (1) the length of time the seeds had been stored (at least 22 years); (2) the number of geographic seed sources available for the test; and (3) the question regarding the best method for storing ponderosa pine seed.

Test II

Twenty geographic seed sources were selected for the second test (fig. 1). Other source data are presented in Read (1980). The selection of sources was made to provide: (1) a range of geographic distribution of sources and (2) a range of seed viabilities as determined by the aforementioned germination test of seeds stored at 5°C. Insufficient seeds precluded use of some seed sources.

Three identical tests (replicates) were run at different times in the spring and early summer of 1987. Three hundred seeds of each source were used (100 seeds for each of three tests). The procedures for a given test involved: (1) use of four plastic (100 mm square, 15 mm deep) petri dishes for each source with 25 seeds in each dish, and (2) incubation of seeds at 24°C under continuous light for 21 days. Petri dishes, germination paper, and distilled water were sterilized to reduce contamination by fungi. Prior to incubation, the seeds were soaked in 30% hydrogen peroxide for 10 minutes to reduce microbial contaminants on the surface of the seeds. The same incubator was used for all three tests. Germination counts were made daily until after day 4, then every other day until germination ceased. Data were evaluated graphically and by analysis of variance.

Results and Discussion

There was no significant difference (P = 0.05) between the two storage temperatures in the percent germination of the seeds of 20 sources of ponderosa pine in the combined data—that is, between seed stored for 22 years in a refrigerator at 5°C and seed stored for 22 years in a freezer at –16°C. Overall germination percentage of the seed stored at –16°C was 64.0%; germination of the seed stored at 5°C was 64.4%. Viability among seed sources ranged from 22% to 80% for seed stored at 5°C, and 34% to 83% for seed stored at –16°C (table 2).

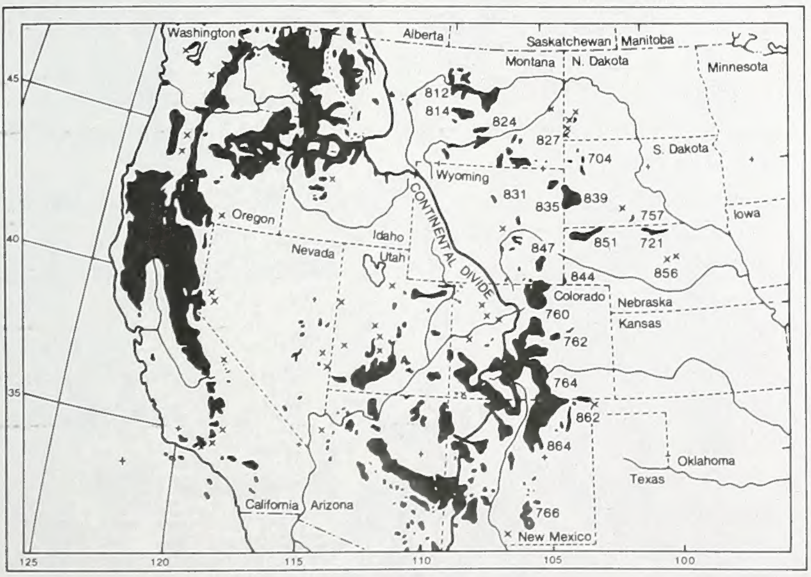

Figure 1.—Natural distribution of ponderosa pine (Little 1971) and locations of seed sources used in seed germination test.

Table 2.—Percentage germination of seeds of geographic sources of *Pinus ponderosa* stored for 22 years at different temperatures.

Seed origin no.	Germination %[a] Seed storage temperature 5°C	-16°C	2-tail prob.
766	79.7	72.7	0.342
864	76.3	47.0	.024*
862	74.7	63.3	.098
764	55.0	69.7	.116
762	66.3	54.6	.108
760	79.0	73.0	.315
844	22.0	56.7	.018*
856	41.0	34.3	.386
847	31.7	49.7	.086
851	62.3	44.3	.143
721	65.7	65.3	.979
757	34.0	69.0	.008*
835	78.3	63.7	.062
831	73.3	60.7	.219
839	73.7	83.3	.008*
704	59.7	67.3	.624
827	79.3	81.3	.742
824	17.3	79.7	.369
814	78.0	65.3	.009*
812	81.3	79.3	.797
Overall	64.43	64.01	

[a] *Basis: 300 seeds (100 seeds in each of 3 tests); total germination after 21 days.*

* *Means are significantly different at the P = 0.05 level of probability based on a t-test computed for each source.*

There was a small but consistent pattern of earlier germination of all sources of seed stored at –16°C during the first 4 to 6 days after incubation (fig. 2). After day 7, germination slowed markedly in both storage temperatures, and germination was essentially completed by day 21, with germination percentages of both temperatures statistically equal (fig. 2). Earlier germination of the seed stored at –16°C through day 6 was consistent in all three tests (replications). However, there was a significant difference (P = 0.01) among the three tests (replications) in the final germination percentages and inconsistency as to whether 5°C or –16°C produced higher germination (interaction between replication and storage was significant, P = 0.06).

In Test A, there was virtually no difference in total germination between the two storage temperatures (66.4% at –16°C, 65.8% at 5°C). In Test B, there was a slight gain in percent germination for the seed stored at –16°C (66.2% vs. 63.7%). In Test C, there was a somewhat greater advantage in the final germination percent for the seed stored at 5°C (63.8% vs. 59.4%). Differences among the three tests are attributed to experimental error or, perhaps in part, to the lower viability of sources 704 and 831 (–16°C) at days 8 and 9 in Test C (fig. 3).

However, the array of seed source viability percentages, illustrated in figure 3, reflects the significant variation (P = 0.01) among the sources and inconsistent response to storage temperature of different sources because the interaction between source and storage temperature was significant (P = 0.01). For example, germination of seeds of source 856 was low, but germination of seeds of sources 839, 827, and 824 was high regardless of storage temperature. Differences between storage temperatures are apparent among sources 864, 844, 757, 839, and 814. Sources 862, 764, 762, 847, 851, 835, and 831 show differences between storage temperatures of a lesser magnitude; sources 766, 760, 856, 721, 704, 827, 824, and 812 show even less difference between storage temperatures (fig. 3).

Except for a few sources of relatively low viability (856, 847, and 851) in the middle latitudes, there does not appear to be an obvious latitudinal pattern (fig. 3). Also, whether or not a seed source is consistent in its germination response and how storage at –16°C compares to storage at 5°C do not appear to be related to latitude.

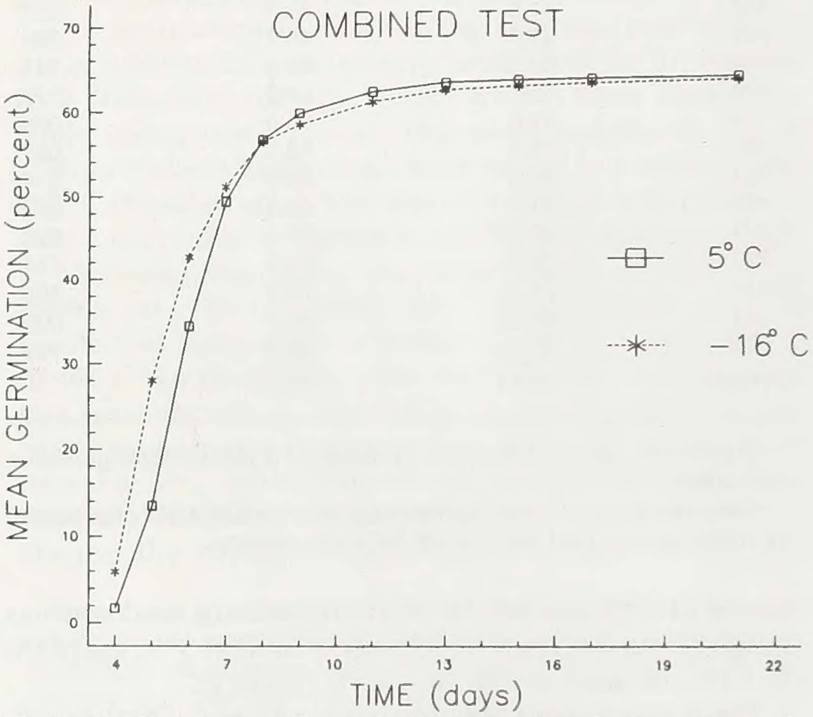

Figure 2.—Mean percent germination over time in three tests of 20 ponderosa pine seed sources stored for 22 years at -16°C and 5°C.

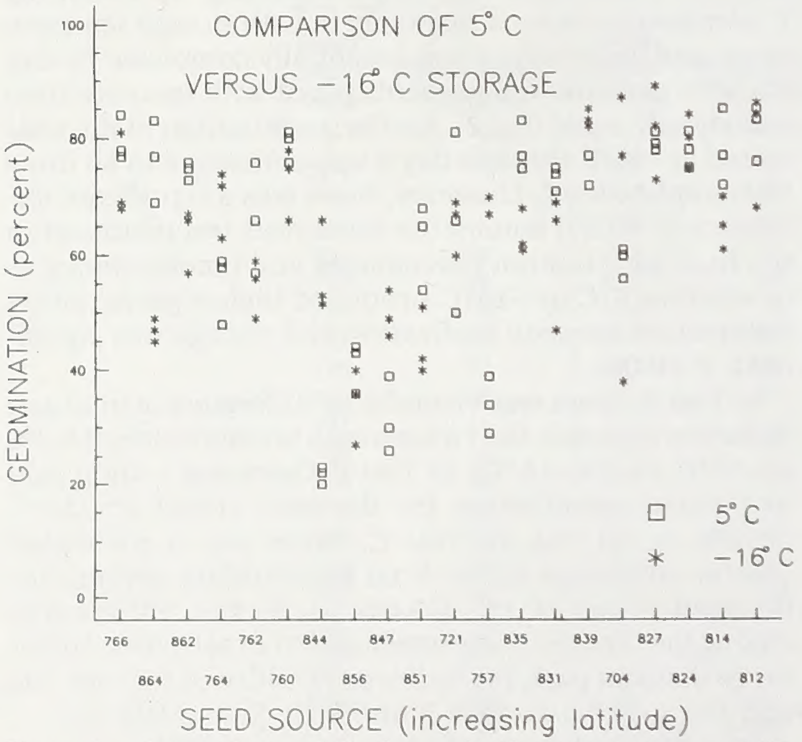

Figure 3.—Comparison of final germination percentages of 20 sources of ponderosa pine stored at -16°C and 5°C, showing variation in the viability of the seed sources, and storage temperature by seed source interaction.

Conclusions

For long-term storage of a variety of seed sources of ponderosa pine from different geographic areas, dry storage at either –16°C in paper sacks or 5°C in glass jars would be suitable. For some individual sources, storage at 5°C would be better. The ability of ponderosa pine to germinate satisfactorily after 22 years of dry storage at 5°C is in accord with findings by Schubert (1952, 1954). The rate of germination, considered over all sources, was initially higher (through days 4 to 6) for seeds stored at –16°C; however, by day 7, the numbers of seeds that had germinated were similar for both storage temperatures. Consistency of germination response and differences between storage at –16°C and storage at 5°C did not appear to be related to latitude.

Literature Cited

Barnett, J. P.; McLemore, B. F. 1970. Storing southern pine seeds. Journal of Forestry. 68: 24–27.

Barton, L. V. 1961. Seed preservation and longevity. New York: Interscience Publishers, Inc. 216 p.

Little, E. L., Jr. 1971. Atlas of United States trees. Vol. I. Conifers and important hardwoods. Misc. Publ. 1146. Washington, DC: U.S. Department of Agriculture. 9 p. with base maps.

Read, R. A. 1980. Genetic variation in seedling progeny of ponderosa pine provenances. Forest Science Monograph 23. 59 p.

Read, R. A. 1983. Ten-year performance of ponderosa pine provenances in the Great Plains of North America. Res. Pap. RM-250. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 17 p.

Schubert, G. H. 1952. Germination of various coniferous seeds after cold storage. Res. Note 83. Berkeley, CA: U.S. Department of Agriculture, California Forest and Range Experiment Station. 7 p.

Schubert, G. H. 1954. Viability of various coniferous seeds after cold storage. Journal of Forestry. 52: 446–447.

Schubert, G. H.; Adams, R. S. 1971. Reforestation practices for conifers in California. Sacramento, CA: State of California Department of Conservation, Division of Forestry Report. 359 p.

U.S. Department of Agriculture, Forest Service. 1974. Seeds of woody plants in the United States. Agric. Handb. 450. Washington, DC: U.S. Department of Agriculture. 833 p.

Van Haverbeke, D. F. 1986. Genetic variation in ponderosa pine: a 15-year test of provenances in the Great Plains. Res. Pap. RM-265. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station. 16 p.